

PCT

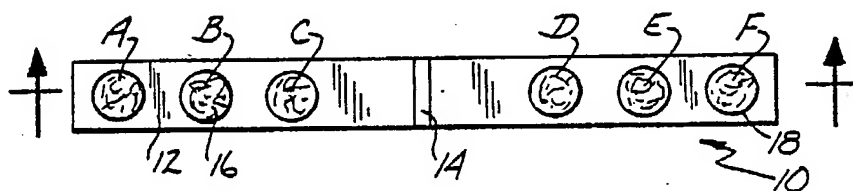
WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification<sup>4</sup> : <b>G01N 33/50, 33/558, C12Q 1/54</b></p>	<p><b>A1</b></p>	<p>(11) International Publication Number: <b>WO 86/ 02165</b> (43) International Publication Date: 10 April 1986 (10.04.86)</p>
<p>(21) International Application Number: PCT/US85/01852 (22) International Filing Date: 30 September 1985 (30.09.85) (31) Priority Application Number: 657,627 (32) Priority Date: 4 October 1984 (04.10.84) (33) Priority Country: US (60) Parent Application or Grant (63) Related by Continuation US 657,627 (CIP) Filed on 4 October 1984 (04.10.84) (71) Applicant (for all designated States except US): BIO-METRICS SYSTEMS, INC. [US/US]; 9932 West 74th Street, Eden Prairie, MN 55344 (US).</p>		<p>(72) Inventors; and (75) Inventors/Applicants (for US only): GUIRE, Patrick, E. [US/US]; 6741 Tartan Drive, Eden Prairie, MN 55344 (US). CHUDZIK, Stephen, J. [US/US]; 3151 Hayes Street N. E., Minneapolis, MN 55418 (US). (74) Agents: BAUMAN, Mary, P. et al.; Fredrikson &amp; Byron, 1100 International Centre, 900 Second Avenue South, Minneapolis, MN 55402 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.  Published With international search report. With amended claims.</p>

(54) Title: FIELD ASSAY FOR LIGANDS



(57) Abstract

Apparatuses and methods which can be used in the field (i.e., outside the laboratory environment) to determine qualitatively and at least semiquantitatively the presence or absence of minute quantities of ligand. The apparatus (10) can be in the form of a strip comprising a support means (12) provided with a groove intermediate its ends forming a crease line (14) upon which the strip can be folded upon itself with bibulous elements (16) and (18) spaced from the crease line and arranged so that when the strip is folded upon itself the bibulous elements become aligned with each other and come into liquid contact.

***FOR THE PURPOSES OF INFORMATION ONLY***

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali		
FR	France				

-1-

FIELD ASSAY FOR LIGANDS  
BACKGROUND OF THE INVENTION

A variety of accurate and reliable assays for measuring minute quantities of analyte dissolved in a solution (e.g., hormones in biological fluids) have been produced and described in the literature. Such assays have commonly required, for their proper performance, a rather high degree of technical and mechanical skill in the measurement of small amounts of reagents, in following detailed procedures, and in using sophisticated analytical equipment. There exists a need for a method of qualitatively and desirably at least semiquantitatively detecting, in the field (that is, outside the laboratory environment) the presence or absence of minute quantities of materials on a rapid basis by persons who often may not be technically skilled. For example, tests for various drug levels in human biological fluids such as urine and blood serum desirably should be available to and capable of use by law enforcement personnel or by paramedics or other emergency medical personnel, inasmuch as it is often of great diagnostic benefit to quickly determine the presence or absence of particular drugs in the blood stream. A ready and effective assay is needed for determining whether certain harmful substances are present in food, such as penicillin

- 2 -

in milk, marine toxins in seafood, etc. Needed also are effective field tests for determining whether pollutants exceed particular concentrations (e.g., salts of mercury in lake water).

The present invention provides apparatuses and methods which are unique in that they can in large measure be readily and easily used or performed in the field by minimally trained personnel.

#### SUMMARY OF THE INVENTION

The present invention provides an apparatus and method for the detection of analyte, the invention making use of first and second normally separated reaction zones, each comprising a bibulous element. Support means are provided to carry the bibulous elements in normally spaced relationship. Means are also provided to establish liquid communication between the bibulous elements. In one embodiment, delay means are positioned between the bibulous elements so that the elements are in a normally spaced relationship, the delay means providing means for establishing liquid-transferring contact between the bibulous elements after a predetermined amount of time has passed.

In one embodiment, a liquid sample suspected of containing an analyte is added to the apparatus and specifically to the first bibulous reaction zone. Broadly speaking, the first reaction zone may contain a chemical system that responds to the analyte, if any, in the liquid sample to provide a liquid-transferable chemical species, the presence or amount of which is related to the presence or amount of the analyte. The apparatus may have two, three or more zones. For example, a liquid analyte sample added to a zone may entrain and carry with it another chemical

- 3 -

species for ultimate liquid transfer to the first bibulous element. As will now be understood, once the bibulous elements are brought together into liquid-transferring contact, the transferrable detectable chemical species, if any, will be transferred to the second bibulous element, resulting in the production of a detectable signal.

Although the apparatus and method of the invention are applicable, in a broad sense, to a wide variety of analytes and chemical detection systems, in its preferred embodiment, (and particularly when small amounts of an analyte are to be detected), the invention makes use of ligand-receptor pairs. The first reaction zone, in this embodiment, may have bound to it one member ("pair member") of a ligand-receptor group comprising, commonly, a pair or set of pairs. Added to the first reaction zone, in addition to the analyte contained in a liquid sample, is a labeled pair member chosen to bind to the first reaction zone in relation to the quantity of analyte in the liquid sample which binds thereto, the label being part of a signal-producing system. The bibulous element of the second reaction zone may carry a label-detection system that is responsive to the label to produce a detectable signal. The invention, in this embodiment, is used by adding to the first reaction zone the liquid sample and the labeled pair member. The presence or amount of the pair member that remains unbound and hence remains liquid-transferrable in the first reaction zone relates to the presence or quantity of analyte in the liquid sample. One or both of the reaction zones are moved in said predetermined path to bring the bibulous elements into liquid-transferring

- 4 -

contact with one another to permit any unbound labeled pair member to transfer to the second reaction zone, the detection system in the latter responding by producing a detectable signal.

A variation of the chemical system described above is found in U.S. Patent 4,446,232 (Liotta). In this patent, ligand-receptor pairs are used, specifically antibody-antigen pairs. The Liotta variation is a noncompetitive assay wherein the immobilized ligand-receptor pair member is the same ligand pair member suspected of being present in the sample. In the instant invention, a ligand-receptor pair member is labeled with an enzyme and is mixed with the sample containing the suspected analyte. The mixture is then added to the first bibulous element in which is immobilized the other ligand-receptor pair member. The enzyme-linked ligand pair member binds with any analyte present in the sample and thus is unable to bind to the immobilized analyte in the first reaction zone. When liquid-transferring contact is established between the first and second reaction zones, the enzyme-linked ligand pair member will move to the second reaction zone containing a material capable of reacting with the labeled antibodies to produce a color-forming reaction which indicates the presence of analyte. The amount or intensity of color corresponds directly to the quantity of analyte in the sample.

#### DESCRIPTION OF THE DRAWING

Figure 1 is a plan view of an apparatus of the invention;

Figure 2 is a cross-sectional view taken along line 2-2 of Figure 1;

Figure 3 is a view of the apparatus shown in Figure 1, folded upon itself;

- 5 -

Figure 4 is a view of a modified apparatus of the invention;

Figure 4A is a broken-away cross-sectional view taken along line 4A-4A of Figure 4;

Figure 5A, B, C and D schematically represent an apparatus of the invention in sequential stages of its use;

Figure 6A, B and C schematically represent another apparatus of the invention in various stages of its use;

Figure 7 is a broken-away, perspective view of a portion of another embodiment of an apparatus of the invention;

Figure 8 is a perspective view of the completed apparatus of Figure 7;

Figure 9 is a cross-sectional view taken along line 9-9 of Figure 8;

Figure 10 is a perspective view similar to that of Figure 8 but taken from the opposite side;

Figure 11 is a broken-away, cross-sectional view similar to that of Figure 9 but showing a step in the use of the device shown therein;

Figure 12 is a diagrammatic view of another embodiment of an apparatus of the invention, suitable for dipping into a liquid sample;

Figure 13 is a perspective view of the device shown in Figure 12 and depicting one step in the analysis process;

Figure 14 is a view similar to that of Figure 13 but showing another step in said process; and

Figure 15 is a view similar to Figures 13 and 14 but showing said steps being carried out simultaneously.

- 6 -

Figure 16 is a broken-away, cross-sectional view similar to that of Figure 11 but showing the use of a delay spacer means.

#### DESCRIPTION OF PREFERRED EMBODIMENTS

As used herein, "ligand-receptor binding pair" or "ligand-receptor pair" refers to a pair of compounds of which one, a "receptor" is capable of recognizing a particular spacial and polar organization of the other ("ligand") or portion thereof, and is capable of binding to that compound. For various ligands, illustrative receptors forming the other half of a ligand-receptor pair include antibodies, enzymes, lectins, Fab fragments, and the like. Commonly, the receptor will be an antibody and the analyte or analyte derivative will act as an antigen or hapten. As used herein, "analyte derivative" means a chemical derivative of an analyte that retains the capacity to bind to the other member of a ligand-receptor pair in competition with the analyte.

By "labeled pair member" or "labeled ligand-receptor pair member" is meant a conjugate of one ligand-receptor pair member with a chemical label such as an enzyme or other detectable chemical species, the conjugate retaining the capacity to bind to the other member of the ligand-receptor pair and the enzyme or other detectable label continuing to have the capacity of being detected by a detector system (which may be a separate chemical reaction system) to provide a perceptible signal. "Detector", "label detector" and the like, refers to a chemical system that provides perceptible signals, commonly electromagnetic radiation or absorption of the same leading to perceptible fluorescence, color changes and



- 7 -

the like, when contacted with a specific enzyme or other label.

Speaking broadly, the apparatus and method of the invention may be used with a large variety of known chemical analysis techniques that involve at least two distinct reactions of which one, in a liquid medium, provides a liquid-transferrable chemical species, the presence or amount of which is related to the presence or amount of analyte in a liquid sample that is added to the apparatus. A separate second reaction involves the detection of the transferrable chemical species to produce a detectable signal.

The invention thus is useful for detecting a broad range of analytes that can be suspended or dissolved in a liquid carrier. Such analytes include inorganic elements and their compounds (usually salts), organic monomers and polymers including macromolecules and assemblages thereof such as subcellular organelles (chromosomes, nuclei, chloroplasts, cell membranes), viruses, bacteria, fungi and other microorganisms. Excellent lists of analytes which are part of specific immunological binding pairs are set out in U.S. Patent 4,374,925 and U.S. Patent 3,817,837, the teachings of which are incorporated herein by reference. Analytes of particular interest include common drugs such as barbiturates and opiates, and various toxins found in food, water and air including natural toxins (microbial, plant, insect, reptilian, etc.) and synthetic (man-made) toxins or poisons. Natural toxins include the marine toxins such as saxitoxin and other paralytic shellfish toxins, ciguatoxin, brevetoxin, palytoxin and the like. Other toxins include mycotoxins (for example, trichothecenes, aflatoxins, patulin, ochratoxins and zearalalone) Synthetic toxins

- 8 -

include nerve agents such as Soman (methylphosphonofluoridic acid, 1,2,2-tri-methylpropyl ester) and Sarin (methylphosphonofluoridic acid, 1-methyl-ethyl ester) and pesticides (e.g., Paraoxon (phosphoric acid diethyl-4-nitrophenyl ester), Furadan, a trademarked product of FMC Corporation, and Malathion, (a product of American Cyanamid)).

In the examples that follow, it will be noted that the analytical reactions by and large fall into two broad groups of which one, applicable primarily to inorganic analytes, involves stoichiometric determinations and the other, applicable primarily to organic materials, involves the use of ligand-receptor binding pairs. In the field of stoichiometric inorganic determinations, the invention is particularly applicable to detecting the presence of an analyte in a concentration above a predetermined concentration. For example, it may be desirable to determine whether an analyte that is a pollutant is present in a concentration above a specific upper concentration that may have been set by a regulatory agency. Inorganic stoichiometric determinations are particularly suitable for analyses that involve volumetric precipitation methods, and compleximetric methods including chelatometric techniques such as may be used for metal ion determinations. In general, such analyses provide for a reaction of the analyte in the sample with a stoichiometrically predetermined amount of reagent to provide a liquid-transferrable species, the presence or amount of which is related to the presence or amount of analyte in the sample.

As to the preferred embodiment of the invention which employs ligand-receptor pairs, reference is made to U.S. Patent 4,391,904 which sets forth a variety of

- 9 -

binding pairs, the teaching of which patent is hereby incorporated by reference. It will be understood that the invention in its broader aspect is not dependent upon the selection of any particular chemical reaction system, but, being in the nature of apparatus and method, is applicable to a variety of such systems.

The "bibulous elements" that are employed herein may be made of filter paper or other fibrous, particulate or porous material that has the capacity to absorb and be wetted by the liquid of the analyte-containing sample. The bibulous elements provide spatially defined and contained reaction zones within which reactions may occur in a liquid environment. The elements hence should not be soluble in the liquid (normally aqueous solutions) containing the sample. Although the elements may swell somewhat, they should be capable of generally holding their shape against substantial deformation even when saturated with liquid. The bibulous elements need not be flexible or compressible. It is desired, however, that at least the first bibulous element be compressible so that, when it is brought into contact with and pressed against the second element, it will tend to decrease in volume and hence liberate liquid which can then be more readily transferred into the second bibulous element. The first bibulous element in the preferred embodiment desirably includes or is made of a reactive material to which a ligand-receptor pair member can be bound. To promote storageability of apparatuses of the invention, the bibulous elements desirably are not reactive in the dry state with any of the reactants that they contain. The second bibulous element desirably is white or is at least light in color so that color changes or other visually perceptible signals

- 10 -

can be readily observed. The bibulous elements may take the form of small discs of filter paper, although fabric, glass wool, polyurethane foams and other materials that can absorb at least small quantities of liquid may be used. The first and second bibulous elements must have exposed or open faces through which liquid may pass when these elements are brought into liquid-transferring contact.

Similarly, the support means which supports the bibulous elements in a normally spaced relationship and which permits them to move in a predetermined path or to otherwise come into liquid-transferring contact with one another desirably is of a material which does not react with either the bibulous elements nor the reactants carried by these elements. Preferably, the support means comprises one or more strips or other appropriate shapes of a polymeric material such as polyethylene or polypropylene, the surfaces of which are generally hydrophobic and are not easily wetted with aqueous solutions. Thus, when a support of the type described carries several bibulous elements, each of which may become saturated with a liquid sample, the tendency of liquid to transfer across the surface of the support from one bibulous element to another is reduced.

The size of apparatuses of the present invention may vary as desired. Commonly, however, the bibulous elements may be discs having diameters of, for example, about 6 mm, and the supports that carry bibulous elements desirably are sized to be held in the hand. Although the bibulous elements carried by the support means may be moved into contact with one another through the use of a mechanical device such as a pair of opposed rollers, in its simpler form the

- 11 -

bibulous elements are supported by the support means in such a fashion as to permit them to be moved, in the predetermined path, by finger pressure, the elements being gently "pinched" together to cause liquid transfer therebetween. As will be evident from the description that follows, the predetermined path followed by one or both bibulous elements desirably is arcuate or straight. In one embodiment, the first and second bibulous strips may be so oriented as to be brought into liquid-transferring contact when one or both of the elements swell upon the addition of liquid thereto.

In another embodiment of the invention, a delay means is employed between the first and second bibulous elements to prevent liquid-transferring contact therebetween until a predetermined amount of time has elapsed. When a liquid sample is added to the first bibulous element, the liquid interacts with the delay means to render it liquid-permeable. The delay means may be embodied in a porous sheet (e.g. filter paper) impregnated or coated with a material which is ultimately dissolved or digested and to establish liquid-transferring contact between the first and second bibulous elements. A variety of coatings may be used, such as gelatin, collagen, triglyceride and tri-olein. Certain materials of this type may be used by themselves to coat one or the other of the bibulous elements. The coating materials may dissolve when liquid is added (e.g., gelatin), or may be digested by an enzyme (e.g., collagen digested by trypsin or collagen digested by collagenase). The digesting enzyme can be bound to the first bibulous element so that it is released when the liquid sample is added or it may be

- 12 -

added in appropriate amounts to the first bibulous element with the sample.

With reference to the drawing, a simple apparatus of the invention is shown in Figures 1-3 and is designated (10). A plastic strip (12), typifying support means, is provided with a groove intermediate its ends forming a crease line (14) upon which the strip can be folded upon itself as shown in Figure 3. To the upper surface of the strip at one side of the crease line (14) are adhered (by means of adhesive tape having adhesive on both surfaces) first bibulous elements (16), typified as filter paper discs. Similarly adhered to the upper surface of the plastic strip at the other side of the crease line (14) are second bibulous elements (18) typified by filter paper discs. The bibulous elements (16) and (18) are so spaced from the crease line (14) and are so arranged that when the plastic strip is folded upon itself as shown in Figure 3, the bibulous elements traverse predetermined paths (arcuate, in this example) and become aligned with one another as shown in Figure 3. Further movement (pinching together) of the elements will bring them into contact with one another, whereupon liquid in the first bibulous elements may be transferred to the second bibulous elements. It will be understood that the apparatus shown in Figures 1-3 can be stored in its unfolded condition within a suitable, desirably moisture-proof, envelope of protective material.

In the simple embodiment shown in Figures 1-3, a typical test may be made for the presence of NaCl in an aqueous solution such as perspiration.

The detection of NaCl present in body fluids such as perspiration in excess of normal levels is

- 13 -

important in the diagnosis of cystic fibrosis. The following example illustrates how the embodiment of Figures 1-3 may be employed to determine the amount of NaCl in a given volume of human perspiration. This example also typifies use of the method and device of the invention with inorganic materials using stoichiometrically controlled amounts of reactants, and makes use of standard chemical techniques (the Volhard determination) for determining the presence of chloride ion.

To bibulous elements A, B and C in Figure 1 are added small quantities of Iron III ammonium sulfate and a predetermined concentration of silver nitrate, except that to bibulous element B is added silver nitrate in substantial excess. Elements D, E and F are identical in that each contains a predetermined concentration of KSCN plus buffer. In addition, bibulous element C is provided with a quantity of sodium chloride in excess of that contained in a given sample volume of normal perspiration.

The test is conducted as follows. Small but predetermined sample volumes of human perspiration containing NaCl are added to each of bibulous elements A, B and C. Chloride ion in the perspiration reacts stoichiometrically with the silver ion, yielding AgCl as an insoluble precipitate; all of the chloride ion in element B, of course, is precipitated due to the excess of silver nitrate in that element. The volume of perspiration added to each of the bibulous elements is enough to thoroughly wet the element and preferably to substantially saturate it.

The apparatus is then folded upon itself as shown in Figure 3, and the opposing bibulous elements are pinched together to, permit fluid flow between

- 14 -

them. As a result (with reference to bibulous elements A and F), fluid transfer causes the ferric ammonium sulfate indicator and any unreacted silver ion to transfer to bibulous element F wherein the silver ion reacts stoichiometrically with the KSCN to yield AgSCN. Any remaining SCN ion reacts with the ferric ion, forming the red  $\text{Fe}(\text{SCN})^{2+}$ . By adjusting the silver nitrate concentrations such that the normal physiological concentration of chloride ion in perspiration precipitates a given amount of silver nitrate yielding a known concentration of free silver ion, and adjusting the KSCN concentration to consume this excess silver ion, the test is adjusted so that the sample containing a normal level of sodium chloride will provide no colored response, whereas the sample with an elevated concentration of sodium chloride will provide a dark red response. Concurrently, the previous addition of NaCl to the bibulous element C provides a red signal in the bibulous element D regardless of the amount of sodium chloride in the patient's perspiration, indicating that the test is operable for large quantities of sodium chloride. Because of the large excess of silver nitrate in element B, no color can form in corresponding element E. Thus, the formation of red signal in element D and of no color in element E provide an indication that the apparatus is operable.

The simple embodiments of Figures 1 through 3 may also be used by an agricultural producer to detect the presence of potato virus in plant extracts. This example typifies use of the method and device of the invention using the labeled antibody-immobilized analyte chemistry described in U.S. Patent 4,446,232 (Liotta).



- 15 -

Potato Virus X (PVX) is a large, nematodic virus, which makes it quite immobile in most assay formats. It is, however, readily degraded into small, globular protein sub-units (D/PVX) by simple exposure to certain known chemicals. In this assay, D/PVX and PVX virus are immobilized onto bibulous elements A, B and C in Figure 1 which comprise discs of filter paper. These discs were prepared as follows: Approximately 6.3 millimeter diameter discs of Whatman #17 chromatography paper were activated by reacting them with CDI as described above, followed by washing with cold borate solution as also described. 1.0 ml. of the D/PVX and/or PVX was added to 100 of the thus prepared discs in a 35 ml. 0.1M borate (pH 9.0) solution and reacted for 20 hours at 4°C. The discs were then washed several times with cold PBS and were stored in PBS at 4°C. The 100 discs were blotted lightly and transferred to a lyophilization flask to which was added 2 ml of a 4 mg/ml albumin in 0.5% PEG 4000/PBS solution. The discs were frozen at -70°C and lyophilized.

The bibulous elements E, F and D each were prepared as follows: Approximately 6.3mm discs of Whatman brand #17 chromatography paper were dried at 105°C for an hour, cooled in a desiccator, and then transferred to a large tube. To the tube was added one gram of 1,1-carbonyldiimidazole ("CDI") and 35 ml. of dry dioxane, and the discs were rocked for 45 minutes at room temperature. The discs thereafter were washed several times with cold deionized water and again several times with cold 0.1M borate solution, pH 9.0. A solution of 35ml of the borate solution containing 10mg of Horseradish Peroxidase was added to the reaction tube and rocked in contact with

- 16 -

the discs for 20 hours at 4°C. The discs then were washed several times with cold phosphate-buffered saline solution ("PBS"), and were then stored in PBS at 4°C. A solution of 10% B-D-Glucose, 0.5% polyethylene glycol ("PEG") 4000, and one mg/ml 2,2'-azinodi-(3-ethylbenzthiazoline sulphonic acid) was prepared, and 20 microliters of the solution was pipetted onto each of the discs prepared above. The discs were then frozen at -70°C and lyophilized.

Glucose oxidase (G.O.) was coupled to antibodies to Potato Virus X ("anti-PVX") by both stepwise glutaraldehyde activation and periodate activation of the enzyme in the following manner:

Dissolve glucose oxidase 100 mg in 8 ml of 20 mM acetate buffer pH5. Add 1 ml 0.2 M  $\text{NaIO}_4$ . Stir four hours in the dark at 4 degrees Centigrade. Add 100 ul ethylene glycol. Step 5: Stir 30 minutes at 4 degrees Centigrade. Dialyze overnite against the following: 25 mM phosphate buffer, pH6.

Dialyze 4.5 ml of  $(\text{NH}_4)_2 \text{SO}_4$  precipitated antiserum against the following: 0.2 M borate buffer, pH 9.5, 0.5 M NaCl, 0.1 M  $\text{NaBH}_3\text{CN}$ . After four hours of dialysis add antibody to the activated G.O. and check pH and adjust to 9.5. Stir overnite at 4 degrees Centigrade.

Add 20 mg of  $\text{NaBH}_4$ . Stir two hours at room temperature. Dialyze against PBS.

These glucose oxidase-antibody conjugates must be affinity purified to prevent non-specific background color development. Conjugates can be prepared by affinity immobilizing antibody onto Sepharose No.

- 17 -

4B-antigen affinity gel and reacting that with glutaraldehyde-activated glucose oxidase. The use of antibody immobilized in this manner permits the protection of its antigen binding sites. The enzyme-antibody conjugate can then be eluted from the 4B-antigen by conventional methods (e.g., 0.1M acetic acid, 3M  $MgCl_2$ , or 1M NaSCN).

The greatest sensitivity can be achieved through the use of monovalent Fab'-G.O. conjugates, since an IgG-G.O. conjugate could theoretically bind both immobilized and free analytes simultaneously. The antibody is first affinity purified on Sepharose 4B-antigen and eluted by conventional methods.  $F(ab')_2$  fragments are prepared from a pepsin digestion of IgG, and Fc fragments are removed on a Protein A column. Disulfide bonds of the  $F(ab')_2$  are reduced (e.g., with cysteine or mercaptoethanol) and Fab' is immediately separated from the reducing agent and reacted with G.O.-maleimide derivative. The conjugate is then chromatographed on a size exclusion column for purification. Again as the G.O.-maleimide is reacting with the free Fab' sulfhydryl group, the antigen binding site is protected.

The test is conducted as follows. Control buffer is mixed with glucose oxidase labeled anti-PVX and is applied to bibulous element A in Figure 1. Analyte mixed with glucose oxidase labeled anti-PVX is applied to bibulous elements B and C in Figure 1. Any available labeled antibody then binds to the PVX immobilized on the bibulous elements A, B and C. The user waits two minutes and then folds the apparatus upon itself as shown in Figure 3, the opposing bibulous elements being pinched together to permit fluid flow between them. As a result (with reference to bibulous

- 18 -

elements A and F), fluid transfer causes the unbound labeled antibody-PVX complex to transfer to bibulous element F wherein the glucose oxidase labeled immune complex reacts with the developing solution contained on the read-out disk.

It would be desirable under some circumstances, e.g., involving drug overdoses, to determine which of several common drugs or drug types have been used. For example, one may wish to detect the presence, in urine or blood serum, of barbiturates (e.g., phenobarbital), opiates (e.g., heroin), or tricyclic antidepressants (e.g., nortriptyline). A suitable apparatus for this purpose is depicted in Figures 4 and 4A, the apparatus utilizing, as in the previously described apparatus, support means in the form of a plastic strip (12) having a crease line (14) intermediate to its ends to permit the strip to be folded upon itself.

As shown, bibulous elements are carried by the plastic strip, the first bibulous elements being designated generally (16) and the second bibulous elements (18). For ease of description, each bibulous element can further be identified by a letter (designating its vertical column) and a number (designating its horizontal row). The bibulous elements (18) are adhered, in the manner described above, to the plastic strip. The bibulous elements (16), however, are carried in apertures (20) formed in the plastic strip (Figure 4A). Additional discs (22) of bibulous material are carried by the apertures (20) adjacent the outer surface of the plastic strip and in liquid-transferring contact with the elements 16. In this manner, urine or other liquid suspected of containing a particular drug may be added to the bibulous elements

- 19 -

(16) from the outer surface (24) (Figure 4A) of the plastic strip, the liquid passing through the bibulous element (22) and carrying reactants stored in that element into the bibulous element (16). The bibulous elements in each of rows 1, 2 and 3 are to be used for detecting the drugs phenobarbital, heroin or a heroin derivative, and nortriptyline, respectively. The elements (16) (18), and (22) are prepared by separately impregnating them with aqueous solutions of reactants and then freeze-drying the elements. One surface of each of the elements (18) is then adhered to the strip (12) as described above, and the elements (16) and (22) may be adhered to the plastic strip by means of small pieces of polyester tape bearing adhesive on both sides. The elements (16) and (22) are carried in liquid-transferring contact with one another, as shown in Figure 4A.

The bibulous elements (18) were prepared in a manner similar to that described above for preparation of disc. D, E and F in Figure 1.

With respect to row 1 (testing for phenobarbital) of the bibulous elements (16), element 1A was prepared in a manner to that described above for discs A, B and C, except that 1.0ml of the anti-phenobarbital IgG fraction of rabbit serum was added to the discs in place of the D/PVX and PVX virus.

Bibulous element 1B was identically prepared, except that 5 micrograms of sodium phenobarbital was added directly to element 1B just prior to the lyophilization step.

The elements (22) used in connection with elements 1A and 1B respectively, were prepared as follows: The sodium salt of phenobarbital was reacted with ethyl-5-bromovalerate, yielding a phenobarbital

- 20 -

derivative with a short spacer arm. The ethyl ester was saponified to the free acid and subsequently esterified with N-hydroxysulfosuccinimide through activation with dicyclohexyl carbodiimide. The thus-activated phenobarbital derivative was coupled to glucose oxidase by reaction in an aqueous solution at alkaline pH. The mixture was then fractionated on a diethylaminoethyl (DEAE)-cellulose column, and the fraction with the preferred combination of high enzyme specific activity and high immunological activity was identified by protein determination, enzyme activity determination and radioimmune equivalence assay. The thus purified glucose oxidase-phenobarbital conjugate is appropriately diluted with 1% PEG and 2 mg./ml. ovalbumin in PBS and applied to filter paper discs, the discs then being freeze-dried at  $-70^{\circ}\text{C}$ .

The bibulous elements (16) found in rows 2 and 3 for the drugs heroin and nortriptyline were prepared in a similar manner, as were the respective elements 22.

The embodiment of Figures 4 and 4A may be used, as by a police officer or ambulance attendant, by adding a single drop of urine suspected of containing one of the three drugs onto the rear side of each of the positions containing elements (16). The liquid sample dissolves and carries with it into the bibulous element (16) the enzyme-labeled analyte carried by the elements (22).

After two minutes, the plastic strip (12) is folded upon its crease line (14), the bibulous elements (16) traveling in predetermined arcuate paths to come into facing engagement with the respective bibulous elements (18). The contacting elements are pinched together momentarily to transfer liquid from

- 21 -

the elements (16) to the elements (18), following which the apparatus is again opened and the elements (18) are observed for the development of color. In each case, the bibulous elements in column X should develop a dark color indicative of the presence of predetermined amounts of the respective drug in the elements in column B. The presence or absence of color in the discs in column Y indicate the presence or absence of the respective drug in the urine of the patient. If the plastic strip (12) defining the support means is transparent, then the color changes in the elements (18) can be viewed from the outer surface of the plastic strip.

Referring now to the embodiment shown in Figure 5, a strip of plastic typifying the support means again is designated as (12). Two grooves, forming crease lines (14) are provided to divide the strip into three substantially equal length sections, designated X, Y and Z respectively. Apertures are formed in the section "X", and discs containing different, predetermined quantities of the phenobarbital-glucose oxidase conjugate exemplified as (22) in Figure 4A are positioned within the apertures. The elements (16) in section Y are identical to element 1A as described in connection with Figure 4. The elements (18) in section Z are identical to elements 1X and 1Y described above in connection with Figure 4.

The embodiment thus described is used by first bending Section X onto Section Y as shown in Figure 5B so that the elements (22) come into contact with the elements (16). A sample of a unknown liquid, e.g., urine, is then added to each of the elements (22), the liquid flushing the varying quantities of the phenobarbital-glucose oxidase conjugate into the bibulous

- 22 -

elements (16). Section X is then unfolded from Section Y, and after a few minutes, Section Z is folded onto Section Y as shown in Figure 5C, the contacting elements being momentarily pinched together to transfer liquid to the bibulous elements (18). Section Z is then again unfolded, as in Figure 5D, and the elements (18) are observed to see which, if any, change color. Phenobarbital in the urine will cause one or more of the elements 18 to become darkly colored within several minutes in accordance with the amount of this drug in the urine sample and depending upon the quantity of phenobarbital-glucose oxidase in the elements 22.

Referring now to the embodiment of Figure 6, a plastic strip (12), provided with a crease line (14), bears on one side first bibulous elements (A) and (B), identical to the bibulous element described above as (1A) in connection with Figure 4. The strip (12) also bears, on the other side of the crease line, bibulous elements (X), (Y) and (Z), each of which is identical to bibulous element 1Y described above in connection with Figure 4. The bibulous strips (16) are each provided with a separate bibulous element (22) also as described in connection with Figure 4A. The bibulous element (C) contains, instead of anti-phenobarbital antibodies, anti-glucose-oxidase antibodies immobilized thereon in sufficient quantity to bind all of the glucose oxidase phenobarbital conjugate in the adjacent element 22. The elements (22) adjacent elements (A) and (B) contain, however, different quantities of the conjugate with element (B) containing substantially less of the conjugate than element (A). The addition of a small sample of urine suspected of containing phenobarbital to each of the elements (16)



- 23 -

results in the presence of liquid-transferrable phenobarbital-glucose oxidase conjugate in element (A) if only a small concentration of the drug is present. Higher concentrations of the drug will also provide transferrable conjugate in the element (B). However, the presence of liquid transferable conjugate in element (C) depends upon the activity of the anti-glucose oxidase antibody therein. Assuming that the anti-glucose oxidase antibody and the antiphenobarbital antibody become deactivated over time at about the same rate, the transfer of the phenobarbital-glucose oxidase conjugate from element C into element (Z) (as indicated by the resultant color change) signals that the apparatus no longer is properly functioning.

When the plastic strip is folded along the crease line (14) and the respective, facing bibulous elements are momentarily pinched together, approximately equal quantities of liquid are transferred from elements (A), (B) and (C) to respective elements (X), (Y) and (Z). Bibulous element (X) will change color in the presence of even small amounts of phenobarbital in the urine specimen. Larger concentrations of phenobarbital in the urine will cause a color change in element (Y) also.

A particularly preferred embodiment of the invention is shown in Figures 7-11. Plastic strips (30) (32), of which at least the latter desirably is transparent, are positioned in parallel, spaced orientation as shown in Figure 7, and resilient spacers (34) positioned at their ends enable the strips to be pinched resiliently together. Carried on the inner surface of the strip (32) are second bibulous elements (18) of the type described above. Carried on the inner surface of the strip (30) are first bibulous elements

- 24 -

(16), also as described above and aligned with the respective bibulous elements (18). The strip (30) may be provided with apertures through its thickness aligned with the bibulous elements (16), and third bibulous elements (22), also as described above, but shaped to fit snugly within the apertures, are also provided in contact with the bibulous element (16). The chemical reagents, for the purpose of this example, will be considered to be the same as those described above in connection with Figures 4 and 4A. The plastic strips (30), (32) and the spacers (34), together typifying support means, may be enclosed in a flexible, water-proof, sealed enclosure typified by the plastic wrapper shown in Figure 9 as (36), the wrapper being generally tubular and being crimped at its ends (38) to form expandable pleats (40), as depicted generally in Figure 9. The wrapper (36) may be provided with apertures (42) (Figure 8) which, desirably, are larger than the apertures formed through the strip (30) and which are generally aligned with the latter apertures to enable a liquid sample to be added directly to the bibulous element (22) from outside of the wrapper. The wrapper similarly is desirably transparent. A removable cover, preferably a strip of adhesive tape (44), covers the apertures (42) and can be stripped away when access to the apertures is desired. The wrapper (36) desirably is air-tight and waterproof. When the adhesive strip (44) is in place, the wrapper and tape provide a waterproof and vapor-proof enclosure enabling the device to be stored for long periods of time.

As thus described, the device may be employed in a dip test procedure in which the device is to be dipped into a liquid sample such as milk or urine

- 25 -

suspected of containing an analyte. First, the strip of tape (44) is peeled away and discarded and the apparatus is immersed just below the surface of the liquid sample for a period sufficient to enable the bibulous strips (16) to become saturated with liquid. This occurs rapidly, commonly within a second or two. As previously mentioned, the surface of the plastic strips is desirably hydrophobic in nature, and tends to prevent liquid from flowing from one bibulous element to another. The device is removed from the liquid sample, blotted, and is permitted to stand for a few minutes as with the device of Figure 4. Using finger pressure, the strips (30), (32) are momentarily pressed together causing the bibulous elements (16), (18) to traverse predetermined, straight-line paths into contact with one another. Liquid is transferred from the elements (16) to the elements (18). The latter bibulous elements (18) may then be observed through the transparent plastic wrapper (38) and the transparent strip (32), as depicted in Figure 10.

Bearing in mind that pinching of the strips (30), (32) together tends to reduce the air volume within the wrapper (38), and hence would tend to raise the air pressure within the wrapper and to force liquid outwardly through the bibulous elements (22), the wrapper desirably is provided with pleated ends or with other expandable structure so that when the elements are pinched together, as shown in Figure 11, the pleat (40) or other structure expands (such expansion being shown in an exaggerated manner in Figure 11) such expansion avoiding substantial internal pressure increases.

- 26 -

In the method in which the device of Figure 8 is dipped into a liquid sample, it will now be understood that the volume of liquid sample that enters the device is strictly and fairly accurately limited by the ability of the elements (16) and (22) to absorb liquid; once the elements have been saturated with liquid sample, no further liquid enters. Also, when the bibulous elements (16), (18) are brought into contact with one another during the pinching operation, the elements (18), being initially dry, tend to rapidly absorb moisture from the elements (16). Desirably, when the elements (16) and (18) are pinched together, their aggregate volume is greater than the non-compressed volume of the element (16). In this manner, the leakage of liquid from one bibulous element to another is restrained.

If desired, gaskets may be provided on the inner surfaces of one or both of the plastic strips to surround and isolate the respective bibulous element pairs so that when the elements are pinched together, the gaskets prevent the passage of liquid from one bibulous element to a neighboring bibulous element on the same plastic strip. The gaskets desirably are of a compressible material that may be identical to that of the spacers (34), and, as elements of the support means, may serve as means normally spacing the bibulous elements (16), (18) from one another. If desired, the gasket material may be absorbant of liquid; in this embodiment, as the plastic strips (30), (32) are pressed together, the absorbant, resilient gasketing material also would be flattened to some extent, momentarily reducing its ability to absorb liquid and improving its ability to prevent the flow of liquid therethrough. When the force pressing the plastic

- 27 -

strips together is removed, the gasketing material, largely regaining its former shape, would commonly absorb any liquid with which it had come into contact, preventing spread of that liquid to other bibulous elements. As will now also be evident, the spacers (34) and gaskets, if any, may also serve to limit the pinching pressure that can be brought to bear upon the bibulous element (16) and (18).

Although the apparatus of the invention has thus far been described as being capable of manual movement to bring bibulous strips into contact with one another, it will be understood that the bibulous elements can be moved by various other mechanisms as well. For relatively large apparatuses of the type shown in Figure 4, care must be taken to insure that each of the bibulous elements (16) comes into liquid-transferring contact with its related bibulous element (18). For this purpose, a mechanism may be employed to insure that the halves of the plastic strip (12) are uniformly pressed together. Various mechanical devices may be employed for this purpose; for example, one may employ a pair of squeeze rollers through which the folded-up apparatus of Figure 4 may be passed. With reference to the embodiment of Figure 8, the bibulous elements (16) may be made of a material that swells as it absorbs liquid - e.g., a crimped paper or polymeric sponge. Upon addition of a liquid sample, sufficient swelling occurs to bring the elements (16), (18) into contact automatically at the desired time.

Referring now to Figure 15, a simplified device of the invention may be employed also in procedures in which the apparatus is to be dipped into a liquid suspected of containing a particular analyte. In this embodiment, a generally V-shaped strip of plastic or

- 28 -

other material, shown as (50), may be employed as the support. Grooves or the like are provided across the legs of the strip to provide crease lines (52). Bibulous elements (54) and (58) are mounted near the ends of the legs of the device, as shown, and a third bibulous element (56) is mounted at the apex of the "V", the bibulous elements and crease lines (52) being so arranged as to permit the strip legs to bend and carry the bibulous elements (54) and (58) in an arcuate path into contact with the bibulous element (56).

As an example of the use of the embodiment of Figures 12-15, the bibulous element (56) may be identical to the bibulous element (16) shown at location 1A in Figure 4, this element containing bound anti-phenobarbital antibody. The disc (54) may be identical to bibulous element (22) shown in Figure 4A and corresponding to position 1A in Figure 4, this element containing enzyme (glucose oxidase)-labeled phenobarbital. The bibulous element (58) may be identical to element (18) shown at location 1F in Figure 4, this element containing the "readout" detection system described in connection with that bibulous element.

In use, the device of Figure 12 is held as shown and the bibulous element (56) is momentarily dipped into a liquid sample (60) suspected of containing an analyte, in this case, phenobarbital, the sample (60) being, for example, urine. The apparatus of Figure 12 is immediately removed from the liquid sample, bibulous element (56) having become saturated with the liquid, and the bibulous element (54) is then bent over into contact with the element (56), as shown in Figure 13, finger pressure being used to assure liquid transfer between the two elements. Liquid from the

- 29 -

bibulous element (56) enters the bibulous element (54), bringing the enzyme-labeled phenobarbital material into solution, the phenobarbital moiety of the test sample (60) and the enzyme-labeled phenobarbital competing for antibody binding sites carried by element (56). After several minutes, the bibulous element (54) is lifted from the element (56), and the other bibulous element (58) is brought into contact with the element (56), as shown in Figure 14. The elements (56) and (58) are pinched together momentarily, following which the element (58) is unfolded and observed for a color change.

If desired, the bibulous element (54) may first be placed in contact with the bibulous element (56), as described above, and, after a short period of time, the leg of the device supporting the element (58) may additionally be folded down as shown in Figure 15, placing all three of the elements in liquid-transferring contact. In this embodiment, of course, the element (56) is to be held in an aperture formed in its respective support leg so that liquid may flow through the element (56) into the element (58), the latter then being observed to detect a color change.

With further reference to the device of Figure 12, the choice and location of reactants may be varied as desired.

The device shown in Figures 12-15 may readily be used in an organic analysis to determine the amount of glucose in, for example, urine. Referring to Figure 12, the bibulous element (56) may contain adenosine triphosphate ("ATP") in a predetermined amount, and the enzyme hexokinase. Bibulous element (54) contains a detection system comprising a chromogen such as o-dianisidine, glucose oxidase and horseradish

- 30 -

peroxidase as a signal generator that is sensitive to the presence of glucose. Element (58) contains glycerol, Methylene Blue, glycerol kinase and glycerol phosphate dehydrogenase. ATP added to the system causes reduction of the indicator Methylene Blue such that the indicator becomes colorless in the presence of a given, stoichiometrically sufficient quantity of ATP.

The present assay can be used to determine whether the concentration of glucose in a body fluid such as urine or blood is above or below the "normal" range. The detection system in element (54) is capable of detecting even quite small quantities of glucose; hence, the amount of ATP contained in the bibulous element (56) is made sufficient to react with all of the glucose in a test sample assuming the glucose concentration to be at the upper end of the "normal" range. If the concentration of glucose in the liquid sample added to the bibulous element (56) were at the lower end of the "normal" range, however, reaction of that quantity of glucose with the ATP would result in the presence of excess or unused ATP in the element (56). Hence, the quantity of Methylene Blue indicator dye in the bibulous element (58) is made sufficiently large as to tolerate the addition of the "excess" ATP, without becoming completely oxidized to its colorless leuco form. The presence of glucose in the liquid specimen at a concentration below the "normal" range results in the availability of additional ATP in the bibulous element (56), so that the ATP in this bibulous element is sufficient to oxidize the Methylene Blue indicator in the bibulous element (58). It will be understood that adjustments can be made to the concentrations of the various ingredients to allow for



- 31 -

less than ideal transfer of glucose on the one hand or ATP on the other to the respective bibulous elements (54) and (58).

In use, a predetermined volume of liquid such as urine or blood plasma containing glucose is placed upon the bibulous element (56). The glucose reacts stoichiometrically with the ATP thereon in the presence of hexokinase, the reaction resulting in the presence of liquid-transferable excess glucose or excess ATP. The bibulous element (56), it will be noted, extends through the thickness of the strip upon which it is carried. The bibulous elements (54) and (58) are then simultaneously folded downwardly as shown in Figure 15 upon opposite sides of the bibulous strip (56). Desirably, the bibulous elements (54) and (58) are far less absorbent than the bibulous element (56) so that the three reaction zones typified by the bibulous elements (56), (54) and (58) become in effect a substantially saturated single zone. Once equilibrium has been substantially reached, the bibulous elements (54), (58) can be returned to the position shown in Figure 12, and observed for a color change. If the concentration of glucose in the physiological fluid was within the "normal" range, no change in color of the bibulous elements (54), (58) should be noted; that is, the element (54) should remain colorless and the element (58) should remain blue. If the glucose in the sample were below the normal range, then the dye indicator in the element (58) becomes reduced to its colorless leuco colorless form, both of the bibulous elements (54) and (58) then appearing colorless to a viewer. In the event that the glucose concentration was above the normal range, this fact is signaled by

- 32 -

the appearance of a reddish-brown color in the bibulous strip (54).

Plastic material, such as polyethylene, has been typified in the foregoing examples as a suitable material for the support means that carries the respective bibulous elements and permits them to be brought into contact with one another by movement over a predetermined path. Various other materials may be used as well, of course, such materials including metals, coated papers, strips of glass (suitably hinged by an adhesive tape strip or the like, or held in an embodiment such as that shown in Figure 7) and the like.

The device shown in Figure 16 may be used with the chemical assays described above in the following manner. Delay means (70) may be comprised of a porous or permeable element such as filter paper. One face of the filter paper is dipped into a dilute solution of warm gelatin and dried. It is inserted into a device similar to that shown in Figure 16 so that it is positioned between a set of first and second bibulous elements. The gelatin coated side of the filter paper is contiguous to the first bibulous element. A liquid sample can then be applied to the first bibulous element (16) through the conduit (75). As the liquid permeates the first bibulous layer (16), it will begin dissolving the gelatin coating on the delay means. The flow of liquid will thus be temporarily delayed for a reasonable period of time. When the gelatin has sufficiently dissolved, liquid-transferring contact will be established between the first and second bibulous elements through the filter paper.

Thus, the instant invention provides an apparatus and method which can be rapidly used to indicate

- 33 -

the presence, and, if desired, to approximate the amount, of a particular analyte. The apparatuses of the invention are simple to operate, and can be used generally in the field by non-technical personnel having a minimum of training. The internal referencing systems serve to indicate whether a particular test is in fact working. Devices of the invention, suitably enclosed in plastic wrappers or envelopes or the like, are expected to exhibit good storageability even under severe temperature conditions since storage occurs in the dry state. However, the internal referencing system as described may be employed to check the viability of stored products of the invention from time to time to assure their continued utility.

While preferred embodiments of the present invention have been described, it should be understood that various changes, adaptations and modifications may be made therein without departing from the spirit of the invention and the scope of the appended claims.

- 34 -

Claim 1.

Apparatus for the chemical analysis of an analyte through at least one chemical reaction in which a liquid-transferable chemical species, the presence or amount of which is related to the presence or amount, respectively, of analyte, is reacted in a liquid medium with a detector system to produce a perceptible signal indicative of the presence or amount of analyte, the apparatus comprising first and second bibulous elements, resilient support means carrying said bibulous elements in a normally spaced relationship, and means for establishing liquid communication between the bibulous elements, thereby enabling any liquid-transferrable species present in the first bibulous element to transfer to the second bibulous element and react with a detector system carried by the second element to produce a perceptible signal.

Claim 2.

Apparatus for the chemical analysis of an analyte comprising:

- A. a first bibulous element containing a chemical reaction system responsive to the addition of an analyte-containing liquid sample to the apparatus to provide a liquid-transferable chemical species, the presence or amount of which is related to the presence or amount, respectively, of analyte in the liquid sample;
- B. a second bibulous element containing a detection system responsive to the liquid-transferable chemical species to produce a perceptible signal; and

- 35 -

C. support means carrying said bibulous elements in a normally spaced relationship but enabling one or both of the bibulous elements to move in a pre-determined path to bring the elements into liquid transferring contact, thereby enabling any transferable chemical species to be transferred to the second bibulous element and reacted with the detection system to produce a perceptible signal.

Claim 3.

The apparatus of Claim 2 in which the support means supporting the second bibulous element is sufficiently transparent as to enable the perceptible signal to be perceived therethrough.

Claim 4.

The apparatus of Claim 2 wherein the support means supporting the first bibulous element includes conduit means enabling a liquid sample to pass there-through into the first bibulous element.

Claim 5.

The apparatus of Claim 4 including a generally water-proof enclosure having ports therein adjacent the support means and positioned to permit the direct addition of a liquid sample to the first bibulous strip through said conduit means.

Claim 6.

The apparatus of Claim 2 in which the support means includes conduit means extending through its thickness and enabling a liquid sample to pass there-through into the first bibulous element.

Claim 7.

The apparatus of Claim 1 in which the means for establishing liquid communication between the bibulous elements comprises a third bibulous element coated with a liquid-dissolvable material.

- 36 -

Claim 8.

The apparatus of Claim 1 in which the means for establishing liquid communication between the bibulous elements comprises a third bibulous element coated with an enzyme-digestable material.

Claim 9.

Apparatus for detecting an analyte in a liquid sample, comprising first and second separate reaction zones, the first zone including a first bibulous element, a chemical reaction system responsive to the addition of an analyte-containing liquid sample thereto to provide an unbound, liquid-transferable chemical species within the first bibulous element, the presence or amount of which species is related to the presence or amount, respectively, of analyte in the liquid sample, and the second reaction zone including a second bibulous element carrying a chemical reaction system responsive to the transferable chemical species to produce a visually perceptible signal; and support means carrying the reaction zones and spacing the bibulous elements in adjacent, opposed, facing, aligned orientation and enabling the elements to move into liquid-transferring contact with one another.

Claim 10.

The apparatus of Claim 6 wherein the support means supporting the second bibulous element is sufficiently transparent as to enable the visually perceptible signal to be visually perceived there-through.

Claim 11.

The apparatus of Claim 7 in which the support means includes conduit means extending through its thickness and enabling a liquid sample to pass there-through into the first bibulous element.

- 37 -

Claim 12.

Apparatus for the chemical analysis of an analyte, comprising first and second reaction zones, the first zone containing a first bibulous element and including a chemical reaction system responsive to the addition of an analyte-containing liquid sample to the apparatus to provide a liquid-transferable chemical species, the presence or amount of which species is related to the presence or amount, respectively, of analyte in the liquid sample, and a second reaction zone including a second bibulous element containing a chemical detection system responsive to the transferable chemical species to produce a visually perceptible signal, the apparatus including first and second support strips carrying the respective first and second bibulous elements, and connection means connecting the first and second strips and orienting the same in spaced, generally parallel planes with the bibulous elements carried in aligned, facing, and spaced relationship to one another, the support strips and connector means being so constructed and arranged as to enable the support strips to be manually pinched towards one another to bring the respective bibulous elements into liquid-transferring contact, the second support strip being transparent to enable the visually perceptible signal to be viewed therethrough.

Claim 13.

The apparatus of Claim 4 including a third bibulous element carried by the support means and positioned to be contacted by the liquid sample passing through said conduit.

- 38 -

Claim 14.

The apparatus of Claim 2 wherein said chemical reaction system comprises a member of a ligand-receptor pair uniformly bound to the first bibulous element, a labeled ligand-receptor pair member chosen to bind to the first bibulous element in relation to the quantity of analyte in said liquid sample that binds to the first bibulous element, and a label detection system carried by the second bibulous element and responsive to said label to produce a detectable signal.

Claim 15.

A method for detecting an analyte contained in a liquid sample, the method employing:

a) a first reaction zone comprising a bibulous element and having bound thereto a member of a ligand-receptor pair;

b) a labeled ligand-receptor pair member chosen to bind to the first reaction zone in relation to the quantity of analyte in the liquid sample, the label being part of a signal-producing system;

c) a second reaction zone comprising a bibulous element carrying a label detection system responsive to said label to produce a detectable signal;

d) support means supporting said reaction zones in a normally spaced relationship; and

e) means for establishing liquid communication between the bibulous elements; said method comprising:

Adding, to the first reaction zone, the liquid sample containing analyte and the labeled ligand-receptor pair member, the amount of the latter member



- 39 -

remaining unbound and liquid-transferable in said first reaction zone relating to the presence or quantity of analyte in said liquid sample; and thereafter

Establishing liquid communication between the bibulous elements, permitting any unbound labeled ligand-receptor pair member to transfer to the second reaction zone, the detection system in the latter producing a detectable signal in response thereto.

Claim 16.

Method for detecting an analyte in a liquid sample, the method employing:

- a) a first reaction zone including a first bibulous element containing a chemical reaction system responsive to the addition of the liquid analyte-containing sample to the first reaction zone to provide a liquid-transferable chemical species, the presence or amount of which is related to the presence or amount of analyte in the liquid sample,
- b) a second reaction zone comprising a bibulous element containing a detection system responsive to the liquid-transferable chemical species to produce a perceptible signal, and
- c) support means supporting said bibulous elements in a normally spaced relationship but enabling one or both elements to move in a predetermined path to bring them into liquid-transferring contact, the method comprising:
  - 1) Adding to the first reaction zone a liquid sample suspected of containing said analyte;

- 40 -

- (2) moving one or both of the bibulous elements into liquid-transferring contact; and
- (3) perceiving said signal.

Claim 17.

Method of Claim 13 wherein the apparatus includes another second bibulous element also movable along a predetermined path into liquid-transferring contact with the first element, the method comprising contacting said other second bibulous element with the first bibulous element and perceiving a signal therefrom.

Claim 18.

The method of Claim 14 whereon said second elements are moved sequentially into contact with the first bibulous element.

Claim 19.

The method of Claim 14 wherein said second bibulous elements are simultaneously moved into contact with the first bibulous element.

Claim 20.

The method of Claim 14 in which said first bibulous element contains adenosine triphosphate and hexokinase, a second bibulous element contains o-dianisidine, glucose oxidase and horseradish peroxidase and the other second bibulous element contains Methylene Blue, glycerol kinase glycerol and glycerol phosphate dehydrogenase, the method comprising the step of adding the first bibulous element a liquid sample suspected of containing glucose.

Claim 21.

A method for detecting an analyte contained in a liquid sample, the method employing:

- 41 -

a) a first reaction zone comprising a bibulous element and having bound thereto a member of a ligand-receptor pair;

b) a labeled ligand-receptor pair member chosen to bind to the first reaction zone in relation to the quantity of analyte in the liquid sample, the label being part of a signal-producing system;

c) a second reaction zone comprising a bibulous element carrying a label detection system responsive to said label to produce a detectable signal;

d) support means carrying said reaction zones; and

e) means for establishing liquid communication between said bibulous elements; said method comprising:

Adding to the first reaction zone, the liquid sample containing analyte and the labeled ligand-receptor pair member, the amount of the latter member remaining unbound and liquid transferable in said first reaction zone relating to the presence or quantity of analyte in said liquid sample; and

Waiting a predetermined amount of time until said bibulous elements establish liquid-transferring contact with one another, permitting any unbound labeled ligand-receptor pair member to transfer to the second reaction zone, the detection system

- 42 -

in the latter producing a detectable signal in response thereto.

Claim 22.

A method for detecting an analyte contained in a liquid sample, the method employing:

a) a labeled ligand-receptor pair member chosen to bind to the analyte in relation to the quantity of analyte in the liquid sample, the label being part of a signal-producing system;

b) a first reaction zone comprising a bibulous element and having bound thereto a ligand-receptor pair member chosen to bind to the labeled ligand-receptor pair member, said labeled ligand-receptor pair member being mixed with analyte prior to application to the first reaction zone such that they will be removed from said first zone when reacted with analyte passing through said first reaction zone but not removed from said first reaction zone in the absence of such analyte;

c) a second reaction zone comprising a bibulous element carrying a label detection system responsive to said label to produce a detectable signal; and

d) Support means supporting the first and second reaction zones in a normally spaced relationship; and

e) means for establishing liquid communication between the bibulous elements; said method comprising:

Mixing the labeled ligand-receptor pair member with the liquid sample suspected of containing the analyte;

- 43 -

Adding, to the first reaction zone, the liquid sample containing analyte and the labeled ligand-receptor pair member, the amount of the latter member remaining unbound and liquid transferable in said first reaction zone relating to the presence or quantity of analyte in said liquid sample; and thereafter

Establishing liquid communication between said bibulous elements, permitting any unbound labeled ligand-receptor pair member to transfer to the second reaction zone, the detection system in the latter producing a detectable signal and response thereto.

## AMENDED CLAIMS

[received by the International Bureau on 10 March 1986 (10.03.86);  
original claims 1-14 cancelled; claims 15-22 amended; new claims  
23-41 added (12 pages)]

Claim 15 (amended).

A method for detecting an analyte which is one of a ligand-receptor pair and which is contained in a liquid sample, the method employing:

a) a first reaction zone comprising a bibulous element and having bound thereto a member of a ligand-receptor pair;

b) a labeled ligand-receptor pair member chosen to bind to the first reaction zone in relation to the quantity of analyte in the liquid sample, the label being part of a signal-producing system;

c) a second reaction zone comprising a bibulous element carrying a label detection system responsive to said label to produce a detectable signal;

d) support means supporting said reaction zones in a normally spaced relationship but enabling one or both of the reaction zones to be moved in a predetermined path to bring the bibulous elements into liquid-transferring contact.

said method comprising:

Adding, to the first reaction zone, the liquid sample containing analyte and the labeled ligand-receptor pair member, the amount of the latter member remaining unbound and liquid-transferable in said first reaction zone relating to the presence or quantity of analyte in said liquid sample; and thereafter

Moving one or both of said reaction zones in said predetermined path to bring said bibulous elements into liquid-transferring contact with one another permitting any unbound labeled ligand-receptor pair member to transfer to the second reaction zone,

the detection system in the latter producing a detectable signal in response thereto.

Claim 16 (amended).

Method for detecting an analyte in a liquid sample, the method employing:

a) a first reaction zone including a first bibulous element containing a chemical reaction system having dry reactants and which is responsive to the addition of the liquid analyte-containing sample to the first reaction zone to provide a liquid-transferable chemical species, the presence or amount of which is related to the presence or amount of analyte in the liquid sample,

b). a second reaction zone comprising a bibulous element containing a detection system having dry reactants and which is responsive to the liquid-transferable chemical species to produce a perceptible signal, and

c) support means supporting said bibulous elements in a normally spaced relationship but enabling one or both elements to move in a predetermined path to bring them into liquid-transferring contact, the method comprising:

1) Adding to the first reaction zone a liquid sample suspected of containing said analyte;

(2) moving one or both of the reaction zones in said predetermined path to bring said bibulous elements into liquid-transferring contact and manually pinching the bibulous elements together permitting liquid-transferable chemical species to

transfer from the first reaction zone  
to the second reaction zone;

(3) perceiving said signal.

Claim 17 (amended).

Method of Claim 16 wherein the apparatus includes another second bibulous element also movable along a predetermined path to be brought into liquid-transferring contact with the first element, the method comprising contacting said other second bibulous element with the first bibulous element and perceiving a signal therefrom.

Claim 18 (amended).

The method of Claim 17 comprising moving said second elements sequentially into contact with the first bibulous element.

Claim 19 (amended).

The method of Claim 17 comprising moving said second bibulous elements simultaneously into contact with the first bibulous element, the second bibulous elements contacting opposite faces of the first bibulous element.

Claim 20 (amended).

The method of Claim 17 in which said first bibulous element contains adenosine triphosphate and hexokinase, a second bibulous element contains o-dianisidine, glucose oxidase and horseradish peroxidase and the other second bibulous element contains Methylene Blue, glycerol kinase glycerol and glycerol phosphate dehydrogenase, the method comprising the prior step of adding to the first bibulous element a liquid sample suspected of containing glucose.

Claim 21 (amended).

A method for detecting an analyte contained in a liquid sample, the method employing:



a) a first reaction zone comprising a first bibulous element and having bound thereto a member of a ligand-receptor pair;

b) a labeled ligand-receptor pair member chosen to bind to the first reaction zone in relation to the quantity of analyte in the liquid sample, the label being part of a signal-producing system;

c) a second reaction zone comprising a second bibulous element carrying a label detection system responsive to said label to produce a detectable signal;

d) and including an aperture contiguous to the first bibulous element; and

e) a third bibulous element containing the labeled ligand-receptor pair member and carried by the aperture adjacent to the outer surface of the support means and in liquid-transferring contact with the first bibulous element;

f) support means carrying said reaction zones[; and] in a normally spaced relationship but enabling one or both elements to move in a predetermined path to bring the bibulous elements into liquid-transferring contact.

said method comprising:

Adding to the first reaction zone through the third bibulous element, the liquid sample containing analyte, the labeled ligand-receptor pair member, the amount of the latter member remaining unbound and liquid

transferable in said first reaction zone relating to the presence or quantity of analyte in said liquid sample being carried into the first reaction zone by the liquid sample; and

After expiration of a predetermined amount of time moving the first and second bibulous elements in a predetermined path to establish liquid-transferring contact with one another, permitting any unbound labeled ligand-receptor pair member to transfer to the second reaction zone, the detection system in the latter producing a detectable signal in response thereto.

Claim 22 (amended).

A method for detecting an analyte contained in a liquid sample, the method employing:

- a) a labeled ligand-receptor pair member chosen to bind to the analyte in relation to the quantity of analyte in the liquid sample, the label being part of a signal-producing system;
- b) a first reaction zone comprising a bibulous element and having bound thereto a ligand-receptor pair member chosen to bind to the labeled ligand-receptor pair member, said labeled ligand-receptor pair member being mixed with analyte prior to application to the first reaction zone such that the labeled ligand receptor pair member that reacts with analyte will pass through said first zone but will not

pass through said first reaction zone in the absence of such analyte;

c) a second reaction zone comprising a bibulous element carrying a label detection system responsive to said label to produce a detectable signal; and

d) Support means supporting the first and second reaction zones in a normally spaced relationship but enabling one or both elements to move in a predetermined path to bring the elements into liquid-transferring contact;

said method comprising:

Mixing the labeled ligand-receptor pair member with the liquid sample suspected of containing the analyte;

Adding, to the first reaction zone, the liquid sample containing analyte and the labeled ligand-receptor pair member, the amount of the latter member remaining unbound and liquid transferable in said first reaction zone relating to the presence or quantity of analyte in said liquid sample; and thereafter

Moving said bibulous elements in a predetermined path to bring them into liquid-transferring contact permitting any unbound labeled ligand-receptor pair member to transfer to the second reaction zone, the detection system in the latter producing a detectable signal and response thereto.

Claim 23 (new).

Apparatus for the chemical analysis of a analyte through at least one chemical reaction, the apparatus comprising a first bibulous element containing a chemical reaction system including dry reactants and which is reactive with added analyte to provide a liquid-transferable chemical species the presence or amount of which is related to the presence or amount, respectively, of analyte, a second bibulous element carrying a detector system including dry reactants and which is responsive to the liquid-transferable chemical species to produce a perceptible signal, and resilient support means carrying said elements in a normally spaced relationship but enabling one or both of the bibulous elements to move in a predetermined path to bring the elements into liquid-transferring contact.

Claim 24 (new).

The apparatus in Claim 23 in which the support means supporting the second bibulous element is sufficiently transparent as to enable the perceptible signal to be perceived therethrough.

Claim 25 (new).

The apparatus of Claim 23 in which the support means includes an aperture in liquid communication with said first bibulous element.

Claim 26 (new).

The apparatus of Claim 25 including a third bibulous element carried by the aperture adjacent the outer surface of the support means and in liquid-transferring contact with the first bibulous element.

Claim 27 (new).

The apparatus of Claim 26 wherein the third bibulous element carries a dry reactant to be carried into the first bibulous element by the liquid sample.

Claim 28 (new).

The apparatus of Claim 23 wherein the chemical reaction system comprises a first member of a ligand-receptor pair bound in the first bibulous element, a labeled ligand-receptor pair member which through reaction with the analyte is prevented from binding to the first ligand-receptor pair member bound in the first bibulous element in relation to the quantity of analyte in the liquid sample, and a label detection system carried by the second bibulous element and responsive to said label to produce a perceptible signal.

Claim 29 (new).

The apparatus of Claim 23 wherein the chemical reaction system comprises a member of a ligand-receptor pair bound to the first bibulous element, a labeled ligand-receptor pair member chosen to bind to the first bibulous element in relation to the quantity of analyte in the liquid sample and a label detection system carried by the second bibulous element and responsive to said label to produce a perceptible signal.

Claim 30 (new).

Apparatus for detecting an analyte in a liquid sample comprising first and second reaction zones, the first reaction zone including a first bibulous element containing a chemical reaction system having dry reactants and which is responsive to the addition of an analyte-containing liquid sample thereto to provide an unbound, liquid-transferable chemical species within the first bibulous element, the presence or amount of which species is related to the presence or amount, respectively, of analyte in the liquid sample, the second reaction zone including a second bibulous

Claim 34 (new).

The apparatus of Claim 31 wherein the first bibulous element is carried within an aperture formed in the support means.

Claim 35 (new).

The apparatus for the clinical analysis of an analyte, comprising first and second reaction zones, the first reaction zone containing a first bibulous element carrying a chemical reaction system having dry reactants and which is responsive to the addition of an analyte-containing liquid sample thereto to provide a liquid transferable chemical species within the first bibulous element, the presence or amount of which species is related to the presence or amount, respectively, of analyte in the liquid sample, a second reaction zone including a second bibulous element containing a chemical detection system having dry reactants and which is responsive to the transferable chemical species to produce a visually perceptible signal, the apparatus including first and second support strips carrying the respective first and second bibulous elements and connection means connecting the first and second strip and orienting the same in spaced generally parallel planes with the bibulous element carried in aligned, facing and spaced relationship to one another, the support strip being so constructed and arranged so as to enable the support strips to be manually pinched together towards one another to bring the respective bibulous elements into liquid-transferring contact, the second support strip being transparent to enable the visually perceptible signal to be viewed therethrough.

Claim 36 (new).

The apparatus of Claim 35 wherein the first support strip includes an aperture through its thickness aligned with the first bibulous element enabling a liquid sample to pass therethrough into the first bibulous element.

Claim 37 (new).

The apparatus of Claim 36 including a generally water-proof enclosure having a port therein adjacent the aperture in the support means and positioned to permit the direct addition of a liquid sample to the first bibulous element through the aperture.

Claim 38 (new).

The apparatus of Claim 37 including a third bibulous element carried by the aperture in liquid transferable contact with the first bibulous element and adjacent the outer surface of the support strip.

Claim 39.

The apparatus of Claim 36 wherein a removable cover covers the outside surface of the enclosure covering the port providing a generally water-proof and vapor-proof enclosure enabling the apparatus to be stored for a period of time prior to use.

Claim 40 (new).

The apparatus of Claim 35 wherein the chemical reaction system comprises a first member of a ligand-receptor pair bound in the first bibulous element, a labeled ligand-receptor pair member which through reaction with the analyte is prevented from binding to the first ligand-receptor pair member bound in the first bibulous element in relation to the quantity of analyte in the liquid sample, and a label detection system carried by the second bibulous element and responsive to said label to produce a perceptible signal.

element carrying a chemical reaction system having dry reactants and which is responsive to the liquid-transferable chemical species to produce a visually perceptible signal; and support means carrying the reaction zones and spacing the first and second bibulous elements in opposed, facing, aligned orientation and enabling one or both of the reaction zones to move in a predetermined path to bring the bibulous elements into liquid-transferring contact.

Claim 31 (new).

The apparatus of Claim 30 wherein the support means supporting the second bibulous element is sufficiently transparent as to enable the visually perceptible signal to be visually perceived therethrough.

Claim 32 (new).

The apparatus of Claim 31 wherein the first bibulous element in the first reaction zone is carried adjacent to an aperture formed in the support means and a third bibulous element is carried by the aperture adjacent the outer surface of the support means and in liquid-transferring contact with the first bibulous element.

Claim 33 (new).

The apparatus of Claim 31 wherein the chemical reaction system comprises a first member of a ligand-receptor pair bound in the first bibulous element, a labeled ligand-receptor pair member which through reaction with the analyte is prevented from binding to the first ligand-receptor pair member bound in the first bibulous element in relation to the quantity of analyte in the liquid sample, and a label detection system carried by the second bibulous element and responsive to said label to produce a perceptible signal.



Claim 41 (new).

The apparatus of Claim 36 wherein the first  
bibulous element is contained within the aperture.

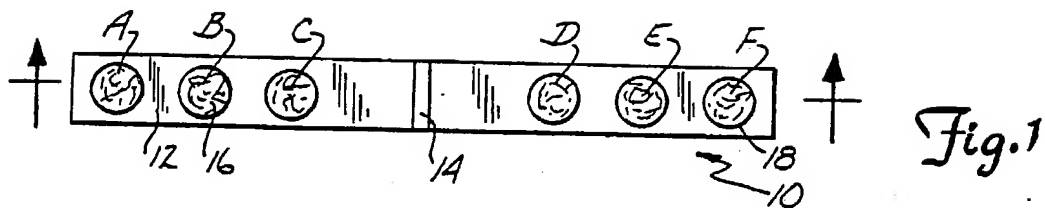


Fig. 1

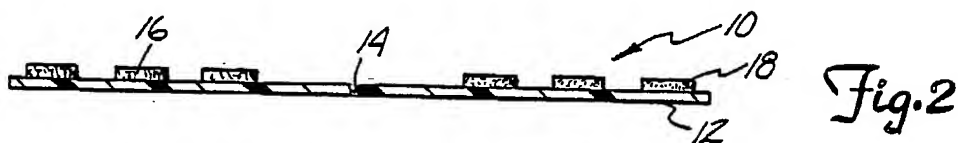


Fig. 2

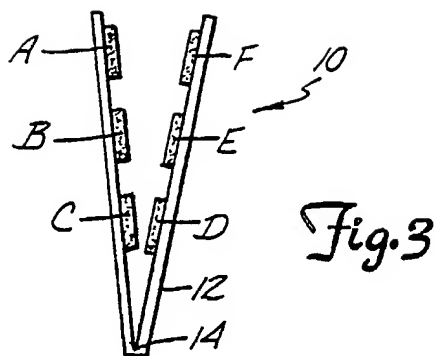


Fig. 3

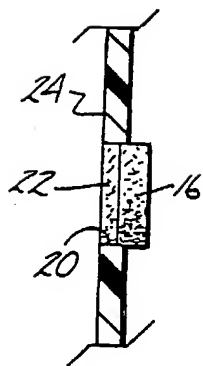


Fig. 4A

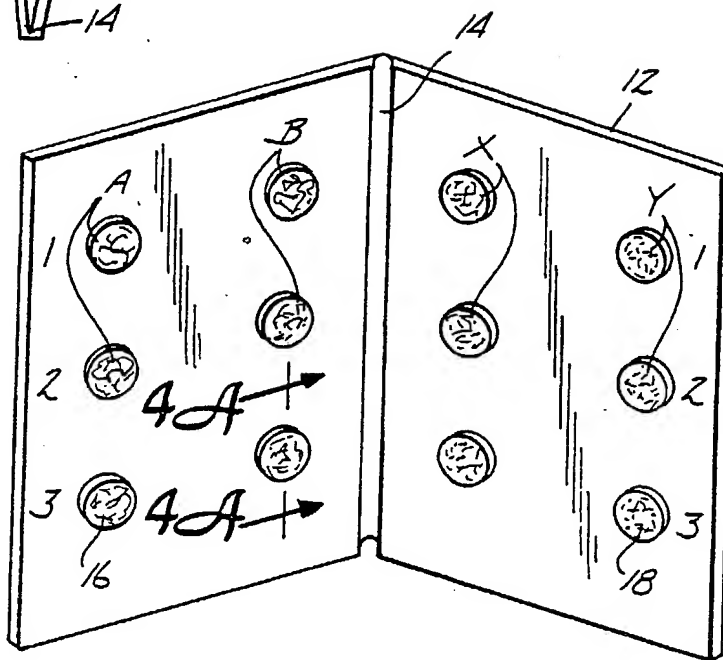
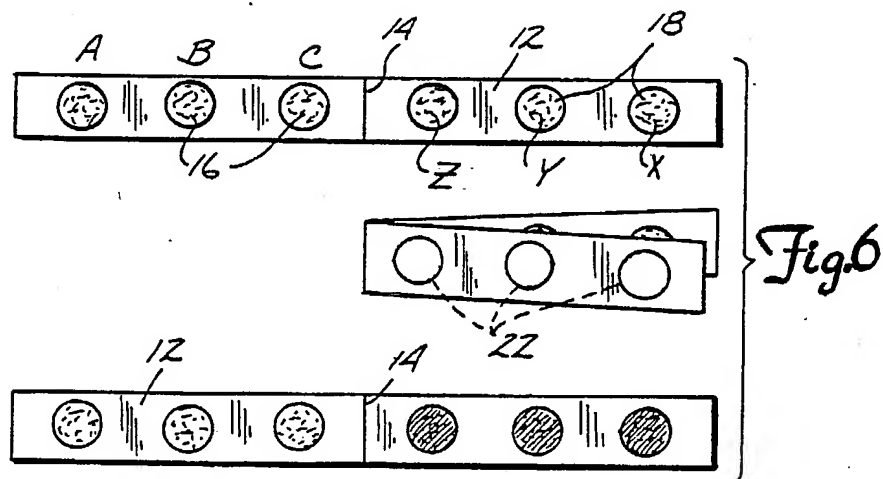
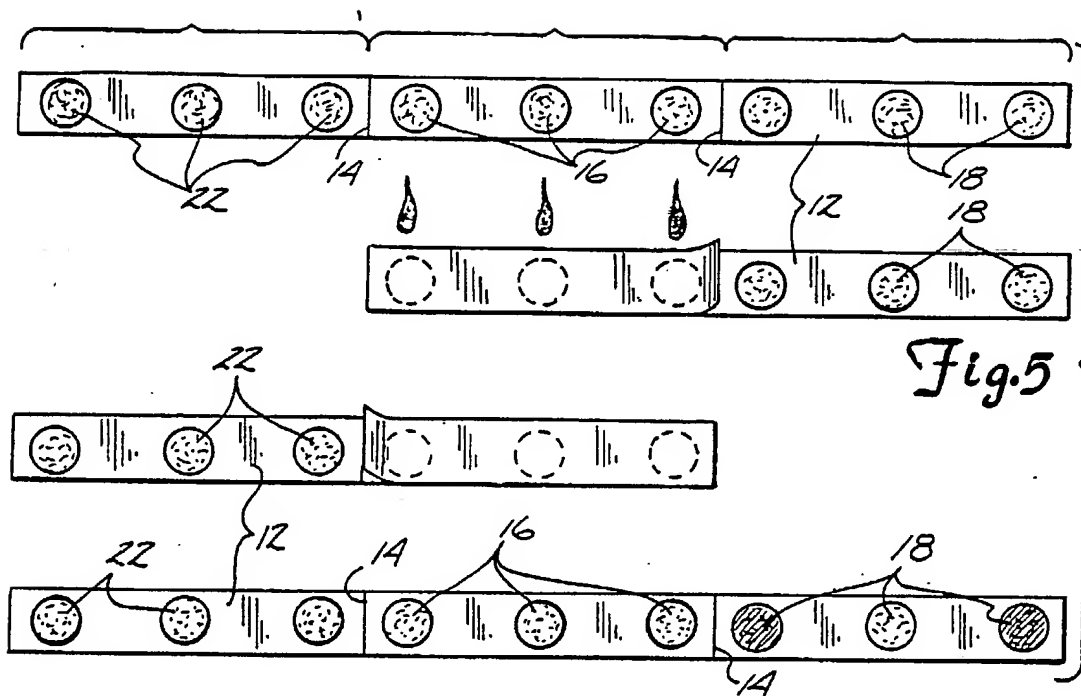
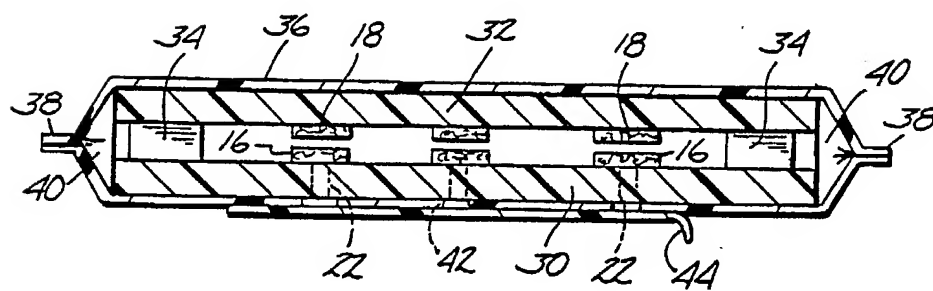
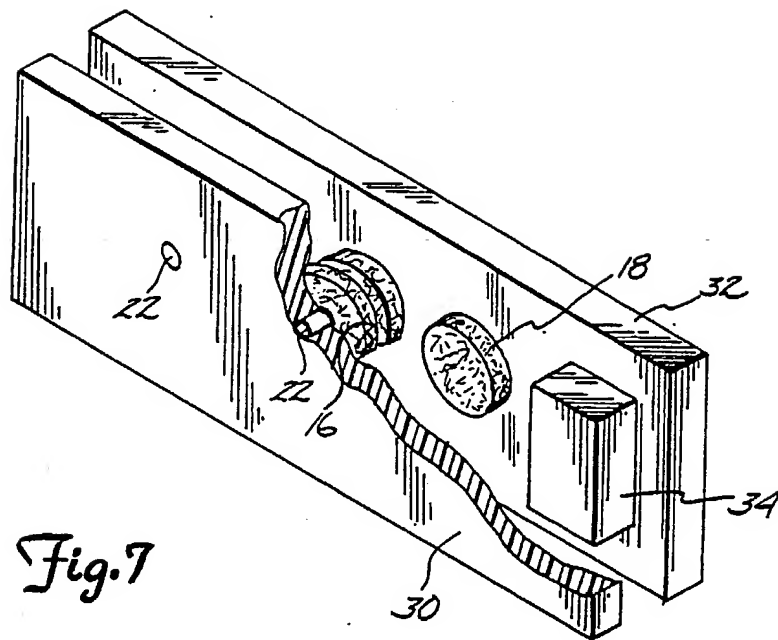


Fig. 4

2/5





4/5

Fig.11

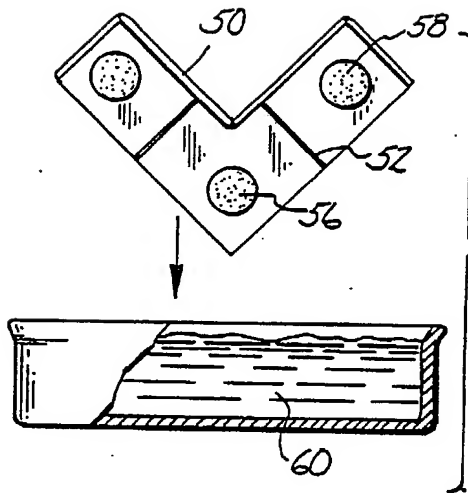
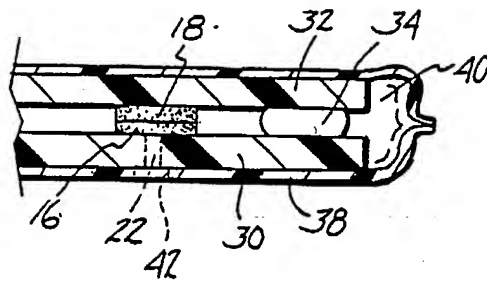


Fig.12

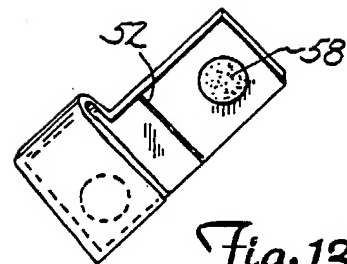


Fig.13

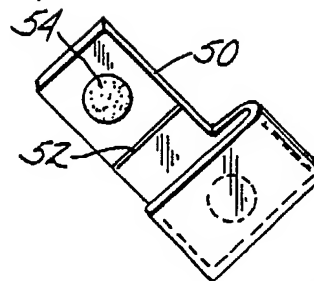


Fig.14

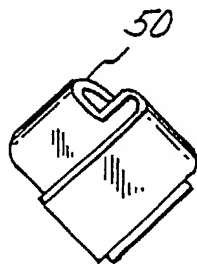
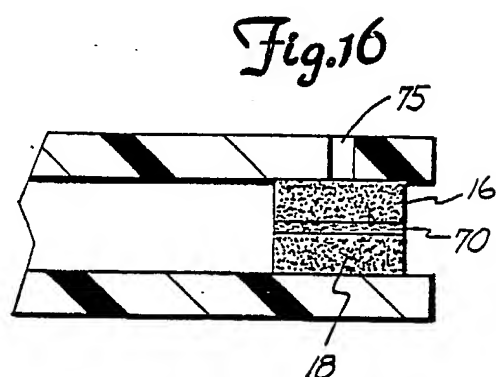
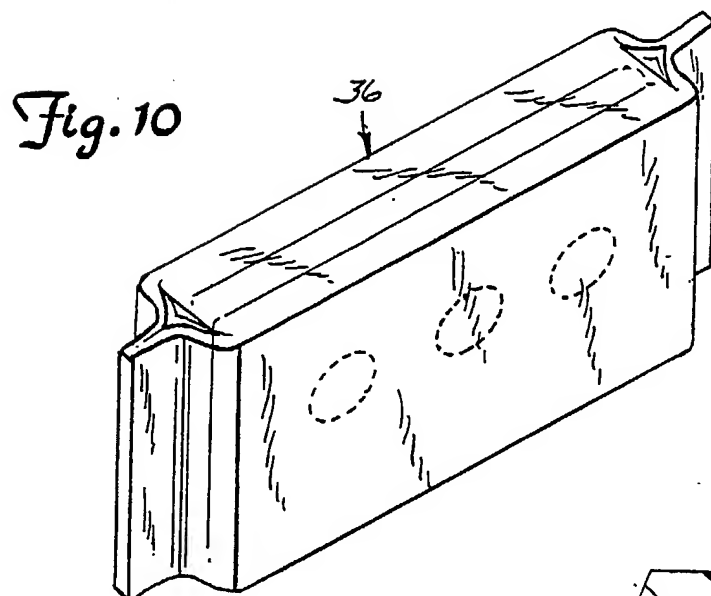
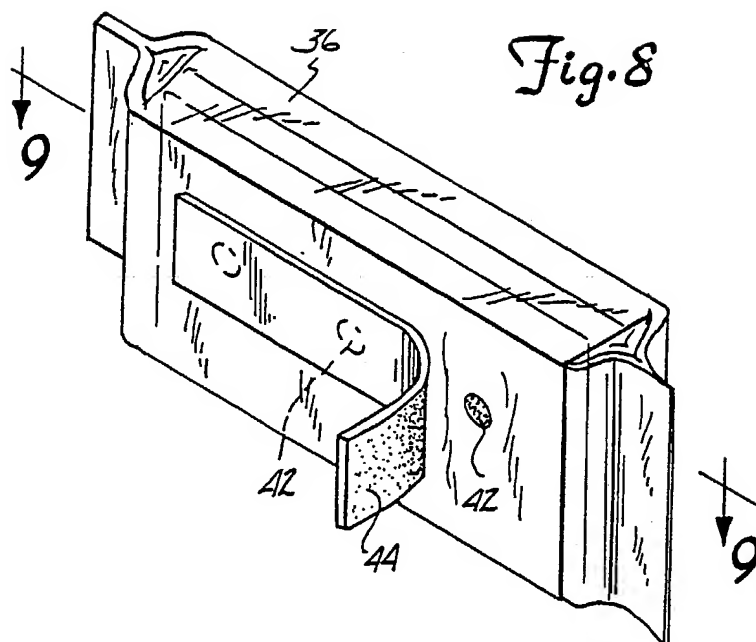


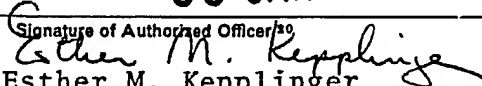
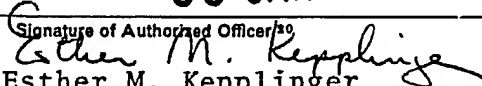
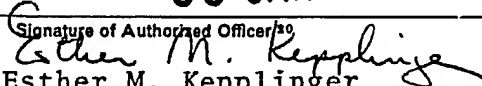
Fig.15

5/5



# INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/01852

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC US- 422/56, 422/58, 436/514, 435/14 IPC <sup>4</sup> -G01N 33/50, G01N 33/558, C12Q 1/54																	
<b>II. FIELDS SEARCHED</b> <table style="width: 100%; border: none;"> <tr> <td style="width: 20%; border: none;"></td> <td style="border: none; text-align: center;">Minimum Documentation Searched <sup>4</sup></td> </tr> <tr> <td style="border: none;"> <table style="width: 100%; border: none;"> <tr> <td style="width: 20%; border: none;">Classification System</td> <td style="border: none;">Classification Symbols</td> </tr> <tr> <td style="border: none; vertical-align: top;">           U.S.         </td> <td style="border: none; vertical-align: top;">           422/56, 57, 58, 61            435/4, 5, 7, 14, 15, 25, 28, 288, 291, 805            436/514, 518, 530, 125, 165, 169, 170, 810, 538, 541         </td> </tr> </table> </td> <td style="border: none;"></td> </tr> <tr> <td colspan="2" style="border: none; text-align: center;">           Documentation Searched other than Minimum Documentation            to the Extent that such Documents are Included in the Fields Searched <sup>5</sup> </td> </tr> </table>				Minimum Documentation Searched <sup>4</sup>	<table style="width: 100%; border: none;"> <tr> <td style="width: 20%; border: none;">Classification System</td> <td style="border: none;">Classification Symbols</td> </tr> <tr> <td style="border: none; vertical-align: top;">           U.S.         </td> <td style="border: none; vertical-align: top;">           422/56, 57, 58, 61            435/4, 5, 7, 14, 15, 25, 28, 288, 291, 805            436/514, 518, 530, 125, 165, 169, 170, 810, 538, 541         </td> </tr> </table>	Classification System	Classification Symbols	U.S.	422/56, 57, 58, 61 435/4, 5, 7, 14, 15, 25, 28, 288, 291, 805 436/514, 518, 530, 125, 165, 169, 170, 810, 538, 541		Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>5</sup>						
	Minimum Documentation Searched <sup>4</sup>																
<table style="width: 100%; border: none;"> <tr> <td style="width: 20%; border: none;">Classification System</td> <td style="border: none;">Classification Symbols</td> </tr> <tr> <td style="border: none; vertical-align: top;">           U.S.         </td> <td style="border: none; vertical-align: top;">           422/56, 57, 58, 61            435/4, 5, 7, 14, 15, 25, 28, 288, 291, 805            436/514, 518, 530, 125, 165, 169, 170, 810, 538, 541         </td> </tr> </table>	Classification System	Classification Symbols	U.S.	422/56, 57, 58, 61 435/4, 5, 7, 14, 15, 25, 28, 288, 291, 805 436/514, 518, 530, 125, 165, 169, 170, 810, 538, 541													
Classification System	Classification Symbols																
U.S.	422/56, 57, 58, 61 435/4, 5, 7, 14, 15, 25, 28, 288, 291, 805 436/514, 518, 530, 125, 165, 169, 170, 810, 538, 541																
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>5</sup>																	
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>14</sup></b> <table style="width: 100%; border: none;"> <tr> <td style="width: 10%; border: none;">Category *</td> <td style="width: 60%; border: none;">Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup></td> <td style="width: 30%; border: none;">Relevant to Claim No. <sup>18</sup></td> </tr> <tr> <td style="border: none; text-align: center; vertical-align: top;">X</td> <td style="border: none; vertical-align: top;">           US,A, 4,446,232 Published            01 May 1984, See Col. 5,            lines 1-28, Liotta         </td> <td style="border: none; text-align: center; vertical-align: top;">1</td> </tr> <tr> <td style="border: none; text-align: center; vertical-align: top;">X</td> <td style="border: none; vertical-align: top;">           US,A, 4,459,358 Published            10 July 1984, See Col. 9,            lines 39-68, Berke         </td> <td style="border: none; text-align: center; vertical-align: top;">1</td> </tr> <tr> <td style="border: none; text-align: center; vertical-align: top;">X</td> <td style="border: none; vertical-align: top;">           US,A, 4,288,228 Published            08 September 1981, See Col.            4, lines 31-41 and Col. 8,            lines 10-19, Oberhardt         </td> <td style="border: none; text-align: center; vertical-align: top;">1</td> </tr> <tr> <td style="border: none; text-align: center; vertical-align: top;">X</td> <td style="border: none; vertical-align: top;">           US,A, 3,644,177 Published            22 February 1972, See Col.            4, lines 44-72, Zyk         </td> <td style="border: none; text-align: center; vertical-align: top;">1</td> </tr> </table>			Category *	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>	X	US,A, 4,446,232 Published 01 May 1984, See Col. 5, lines 1-28, Liotta	1	X	US,A, 4,459,358 Published 10 July 1984, See Col. 9, lines 39-68, Berke	1	X	US,A, 4,288,228 Published 08 September 1981, See Col. 4, lines 31-41 and Col. 8, lines 10-19, Oberhardt	1	X	US,A, 3,644,177 Published 22 February 1972, See Col. 4, lines 44-72, Zyk	1
Category *	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>															
X	US,A, 4,446,232 Published 01 May 1984, See Col. 5, lines 1-28, Liotta	1															
X	US,A, 4,459,358 Published 10 July 1984, See Col. 9, lines 39-68, Berke	1															
X	US,A, 4,288,228 Published 08 September 1981, See Col. 4, lines 31-41 and Col. 8, lines 10-19, Oberhardt	1															
X	US,A, 3,644,177 Published 22 February 1972, See Col. 4, lines 44-72, Zyk	1															
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; vertical-align: top;">           * Special categories of cited documents: <sup>16</sup>            "A" document defining the general state of the art which is not considered to be of particular relevance            "E" earlier document but published on or after the international filing date            "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)            "O" document referring to an oral disclosure, use, exhibition or other means            "P" document published prior to the international filing date but later than the priority date claimed         </td> <td style="width: 50%; border: none; vertical-align: top;">           "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention            "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step            "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.            "&amp;" document member of the same patent family         </td> </tr> </table>			* Special categories of cited documents: <sup>16</sup> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family													
* Special categories of cited documents: <sup>16</sup> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family																
<b>IV. CERTIFICATION</b> <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none;">           Date of the Actual Completion of the International Search <sup>3</sup>  <div style="text-align: center; font-weight: bold;">12 December 1985</div> </td> <td style="width: 50%; border: none;">           Date of Mailing of this International Search Report <sup>3</sup>  <div style="text-align: center; font-weight: bold;">09 JAN 1986</div> </td> </tr> <tr> <td style="border: none;">           International Searching Authority <sup>1</sup>  <div style="text-align: center; font-weight: bold;">ISA/US</div> </td> <td style="border: none;">           Signature of Authorized Officer <sup>20</sup>  <div style="text-align: center;">               Esther M. Kepplinger           </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <sup>3</sup> <div style="text-align: center; font-weight: bold;">12 December 1985</div>	Date of Mailing of this International Search Report <sup>3</sup> <div style="text-align: center; font-weight: bold;">09 JAN 1986</div>	International Searching Authority <sup>1</sup> <div style="text-align: center; font-weight: bold;">ISA/US</div>	Signature of Authorized Officer <sup>20</sup> <div style="text-align: center;">               Esther M. Kepplinger           </div>											
Date of the Actual Completion of the International Search <sup>3</sup> <div style="text-align: center; font-weight: bold;">12 December 1985</div>	Date of Mailing of this International Search Report <sup>3</sup> <div style="text-align: center; font-weight: bold;">09 JAN 1986</div>																
International Searching Authority <sup>1</sup> <div style="text-align: center; font-weight: bold;">ISA/US</div>	Signature of Authorized Officer <sup>20</sup> <div style="text-align: center;">               Esther M. Kepplinger           </div>																

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US,A, 4,459,358 Published 10 July 1984, Berke	2-22
Y	US,A, 4,446,232 Published 01 May 1984, Liotta	2-22
Y	US,A, 4,288,228 Published 08 September 1981, Oberhardt	2-22

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>10</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ..... because they relate to subject matter <sup>12</sup> not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out <sup>13</sup>, specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>11</sup>

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.



## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No <sup>18</sup>
Y	US,A, 3,644,177 Published 22 February 1972, Zyk	2-22
Y	N,Clinical Chemistry, <u>22(8)</u> , issued 1976, Wisdom, See page 1243, 1243-1255	15,21, 22
Y	US,A, 4,254,083 Published 03 March 1981, Columbus	4-6,11